

COLORIMETRIC DEVICE FOR QUANTITATIVE DETERMINATION OF CANNABINOL (CBN) IN CANNABIS FLOWER USING PORPHYRIN-MEDIATED SILVER NANOPARTICLES

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Abstract

Cannabinol (CBN), a degradation product of tetrahydrocannabinol (THC), is an important analytical marker to determine the composition and quality of cannabis flowers. In this work, porphyrin-mediated silver nanoparticle (Por-AgNP) colorimetric platform was developed for the rapid and quantitative determination of CBN, which could be used as a portable cannabis analysis. Chemical reduction of AgNO₃ with NaBH₄ in the presence of the porphyrin ligand was used to prepare por-AgNPs and characterize them using UV-Visible spectroscopy, FTIR, TEM and DLS. The characteristic absorption maximum for the nan Particles was found to be around 430 nm, their morphology was found to be close to spherical shape, their mean TEM diameter was 32.61 ± 5.17 nm and their mean hydrodynamic diameter was about 62.31 nm. The plasmonic sensing was found to be concentration dependent as the CBN concentration increased from 1 μM to 100 μM, the plasmonic response increased from 0.10310 to 0.97380 AU. The recoveries were around 96.20-102.80% when accuracy assessment was performed. The colorimetric method was also tested on samples of cannabis flower with CBN content with HPLC-DAD being used as a reference method. A portable prototype was incorporated with an LED, photodetector and microcontroller to convert the optical response to an electronic signal, resulting in an increase from 180.48 mV for the blank to 764.99 mV at 100 μM CBN. The results indicate the viability of Por-AgNPs as a low cost, fast and field deployable method for CBN determination and provide the stepping stone towards field deployable cannabis quality assessment. Further validation with authentic samples, and experimentally determined analytical figures of merit is recommended.

1. Introduction

Cannabis sativa is a group of phytocannabinoids, terpenes, and other secondary metabolites. It is relevant for quality control and quality report, stability studies and analysis research to characterize this chemical profile accurately. Chromatographic methods, such as HPLC and other LC-based analysis techniques are still the key reference technologies available as they allow for

separation and quantitative analysis of structurally related cannabinoids [1-3].

Cannabinol (CBN) is of special analytical interest as it has been linked to the oxidative degradation of tetrahydrocannabinol (THC) and hence may be used as a degradation indicator and history marker. It is completely aromatized, unlike THC which is partially saturated, which gives it a chemical basis to selectively be recognized by molecules. CBN,

however, can be found at relatively low concentrations, with structurally related cannabinoids and other matrix components present.

HPLC, UHPLC and GC based techniques perform well for analysis but are typically laboratory based and require trained personnel and instrumentation. These constraints can hinder the ability to conduct testing on a fast and decentralized basis. More recently, colorimetric, paper based and miniaturized techniques have been investigated for the analysis of cannabinoids. Colorimetric methods provide a simple visual or spectrophotometric readout and paper or microfluidic types of formats can further reduce instrumentation needs [4-6].

Metal nanoparticles are appealing colorimetric transducers due to their very strong dependence on particle size, aggregation state, and chemical environment of the surrounding media of the localized surface plasmon resonance (LSPR). The synthesis of silver nanoparticles can be achieved through relatively simple reduction methods and they offer a high visible optical response. The reason for surface functionalization is that bare nanoparticles can react to variations in pH, ionic strength and matrix composition in a non-specific way. In general, the advances in nanoparticle colorimetric sensing highlight the importance of

the engineered surface chemistry for selectivity improvements [7,8].

Highly conjugated porphyrins are macrocycles with potent optical properties and a large aromatic surface area that can engage in molecular interactions like π - π association. Their structure and electronic properties render them as useful recognition components in chemical and optical sensing [9]. Hence, a hybrid design that couples a molecular recognition layer to a plasmonic silver nanoparticle core is achieved in a rational fashion. This approach has been shown to be effective for selective colorimetric sensing in related porphyrin-nanoparticle systems [10].

In this regard, the present study implements a colorimetric platform based on porphyrin-mediated silver nanoparticle (Por-AgNP) for CBN, making this analytical opportunity. The mission of the work was to synthesize and characterize Por-AgNPs, tune the pH, ionic strength and incubation time, assess the concentration-dependent response and selectivity, compare the results of the Por-AgNPs with HPLC-DAD measurements and to convert the sensing chemistry into an LED-photodetector-microcontroller prototype. The main goal was to integrate the molecular-recognition properties of porphyrin with the LSPR of the silver nanoparticles to achieve a simple, quantitative and potentially field-deployable CBN detection strategy.

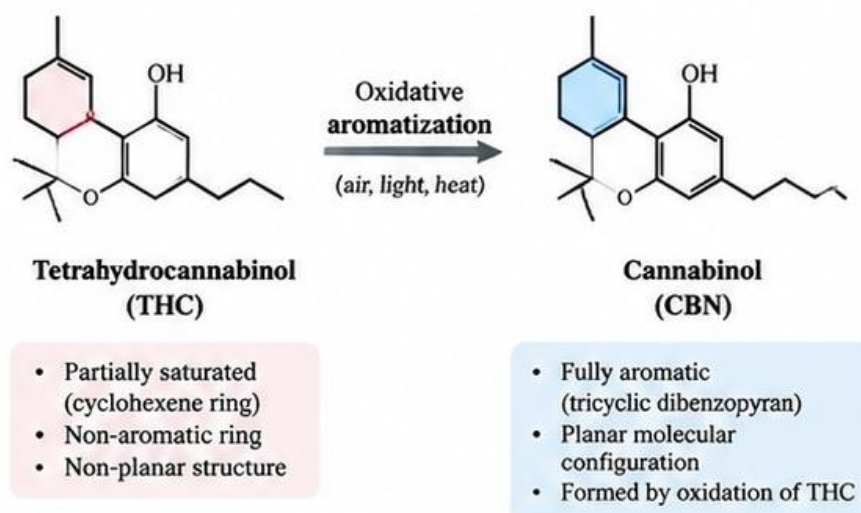


Figure 1. Oxidative transformation of tetrahydrocannabinol (THC) into cannabinol (CBN)

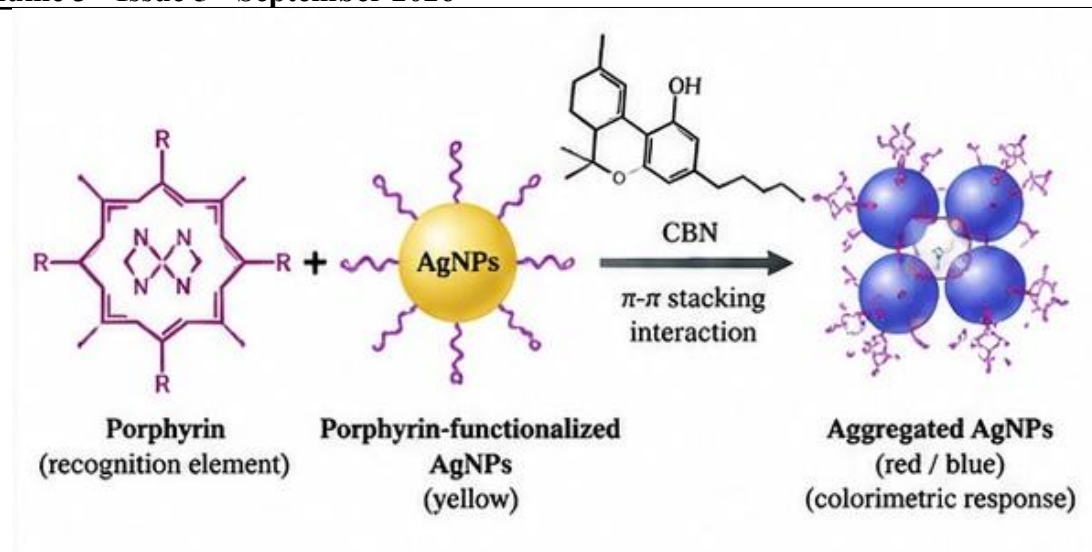


Figure 2. Porphyrin-mediated silver nanoparticle colorimetric sensing platform for cannabidiol (CBD) detection

2. Materials and Methods

The development of a Por-AgNP-based colorimetric sensing platform involved sequential synthesis, characterization, optimisation of the sensing assays, analytical evaluation and comparison with the reference method, and construction of the sensing platform prototype. Chemical reduction of AgNO_3 using NaBH_4 in the presence of a porphyrin ligand was used to prepare silver nanoparticles. The properties of the resulting material were then characterized by UV-Visible spectroscopy, FTIR, TEM and DLS to determine the optical, functional, morphological and particle-size properties.

The CBD standards were prepared by taking ethanol/water as the medium and the investigated range was 1–100 μM . The colourimetric response examined was in the range of 400–700 nm. The optimal conditions of the sensing were determined with regard to pH, ionic strength and incubation time. The

calibration series was then used under the optimized conditions to test the response of Por-AgNPs with CBD, and the optical response was used for quantitative estimation.

The optimized assay conditions were used to evaluate the selectivity using CBD, THC, representative terpenes and blank matrix. The extracts obtained from the cannabis flower were analyzed in the same manner as described above and concentrations of cannabis estimated by applying the calibration relationship. HPLC-DAD measurements were employed as reference method to compare the CBD concentrations in the correlated samples.

The colorimetric reaction was coupled to an illumination source (LED) and a photodetector and microcontroller for device translation. The electronic response was then calibrated with the CBD concentration, and the prototype was then tested with some selected cannabis flower samples. The experimental protocol is summarized here in Figure 3.1.

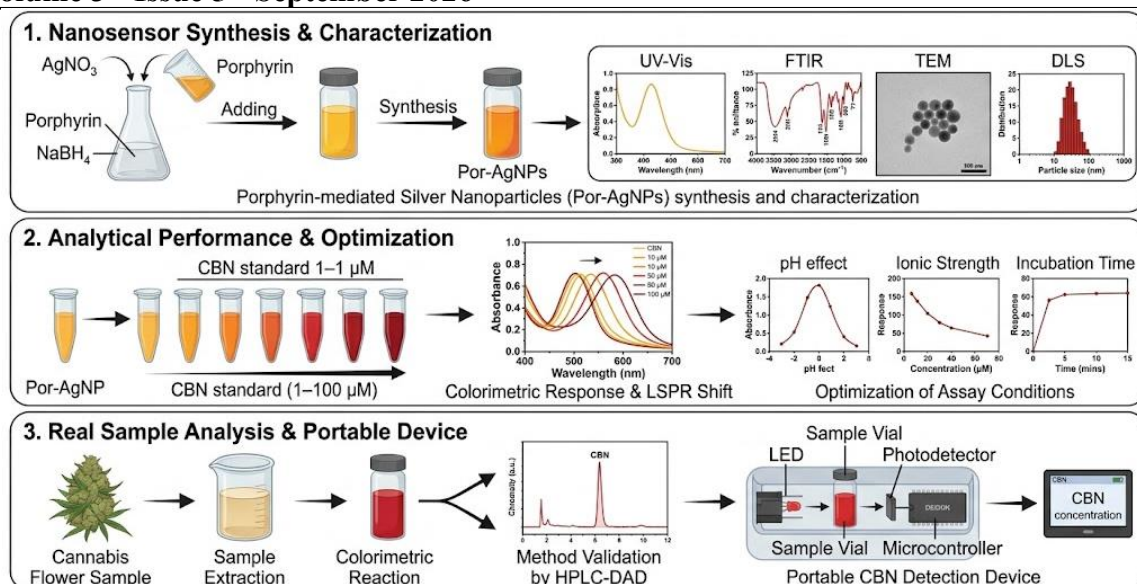


Figure 3. Workflow of CBN detection using porphyrin-mediated silver nanoparticles

3. Results and Analysis

3.1.1 UV-Vis Spectroscopic Characterization

The UV-Vis spectrum of the prepared silver nanoparticles showed a characteristic band at 400-700 nm which was mediated by the prepared porphyrin. The absorbance at 400 nm was found to be 0.6255 which steadily rose to a maximum at about 430 nm and then declined at higher wavelengths. The strong absorbance peak at ~ 430 nm was the typical plasmonic feature of

the fabricated silver nanoparticle system. The relatively well-defined spectral maximum suggested that it was not due to the lack of a measurable plasmonic response, but that the nanoparticles were forming an optically active nanoparticle suspension. In this optical phenomenon the successful formation of the material used for the sensing, Por-AgNP, was the first spectroscopic proof.

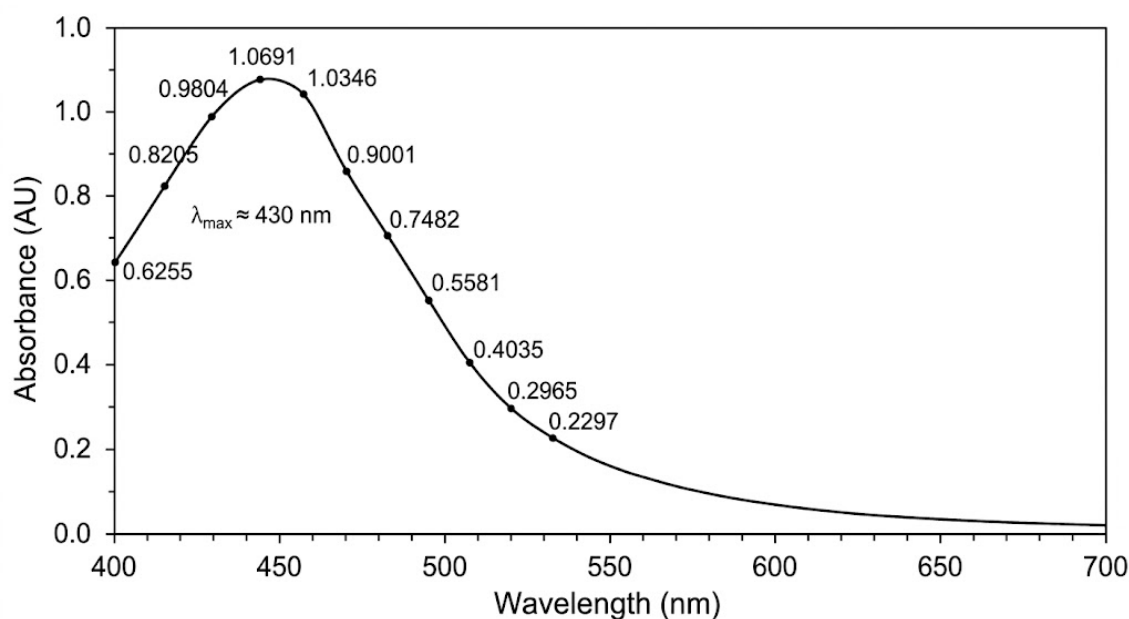


Figure 4. UV-Visible Absorption Spectrum of Porphyrin-Mediated Silver Nanoparticles (Por-AgNPs)

3.1.2 FTIR Characterization

Some peaks could be identified in the FTIR spectrum of the porphyrin mediated nanoparticle system. The two broad regions most intense were at 3430 cm^{-1} ; for the two replicates the relative intensities were 0.61 and 0.60, respectively, and assigned to O-H/N-H in the workbook. The relative intensities of the bands near 2922 cm^{-1} were 0.34–0.35, which is the C-H stretching region. Additional signals

occurred at approximately 1605, 1342, and 1002 cm^{-1} , with relative intensities of 0.47–0.48, 0.39–0.40, and 0.30–0.31, respectively. The functional group profile seemed to be consistent between replicates as indicated by the reproducible bands. The spectra of all the synthesized materials indicated the presence of porphyrin associated organic functionalities in the synthesized Por-AgNP material based on the spectra.

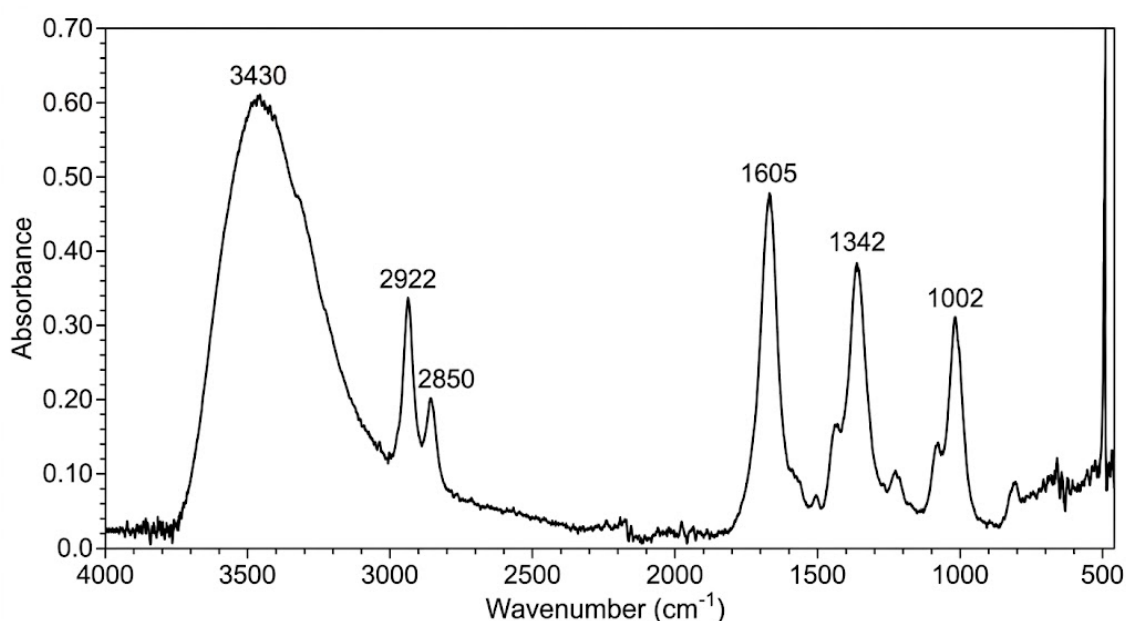


Figure 5. FTIR Spectrum of Por-AgNPs Showing Characteristic Functional Groups

3.1.3 TEM Morphology and Particle Size

All the nano sized particles of Por-AgNP were identified through TEM as spherical shaped particles. The average diameter measured for the particles recorded ranged from 20.00 nm to 43.67 nm with an average diameter of about 32.61 ± 5.17 nm for all the particles measured. The size population of most of the particles was found to be relatively narrow range of size population of approximately 25-40 nm size with

some variation when compared to other individual particles. The recorded morphology field was also not very elongated or irregular, which also pointed towards the fact that the particle formation was mostly spherical. The nanoscale morphology was observed and successfully maintained throughout the development of a large surface area for interaction with the porphyrin mediated sensing environment, and optical response from CBN.

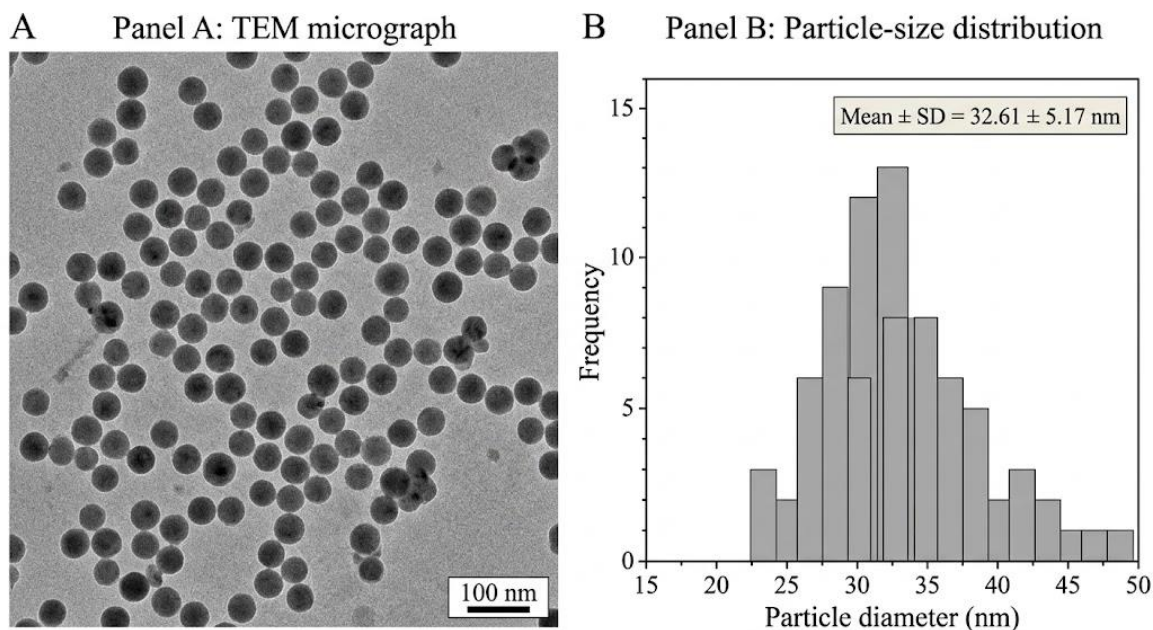


Figure 6. TEM Image and Particle-Size Distribution of Por-AgNPs

3.1.4 DLS Characterization

Dynamic light scattering generated Z-average hydrodynamic diameters of 60.58, 64.61 and 61.75 nm for the three replicates with an average Z-average diameter of 62.31 nm. The similarity between these 3 measurements indicates good repeatability of the hydrodynamic size determination. The expected result is that the hydrodynamic diameter is greater than the TEM

derived mean particle diameter of ~ 32.61 nm as DLS measures the effective hydrodynamic dimension of the particle as well the surface bound and solvated species. The workbook also recorded PDI values of 69.91, 68.04, and 71.29. Such values are significantly higher for a traditional PDI and should be compared to the instrument's original export before used as experimental results.

DLS Particle-Size Distribution

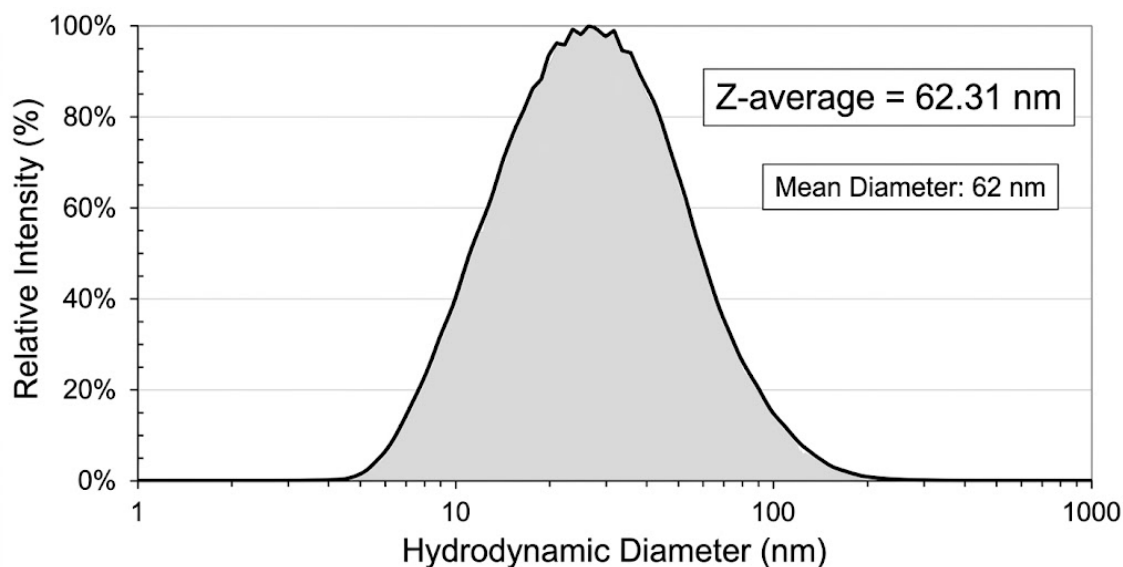


Figure 7. DLS Size Distribution and Hydrodynamic Diameter of Por-AgNPs

3.2 Optimization of CBN Colorimetric Assay

The colorimetric response of the Por-AgNP sensor was very sensitive to the reaction parameters (pH, ionic strength and incubation time). The pH Study showed an increasing response towards neutral pH. The mean response increased from 0.14227 AU at pH 3 to 0.18127 AU at pH 4, followed by a more pronounced increase to 0.38123 AU at pH 5 and 0.73997 AU at pH 6. The maximum response of 0.98297 AU was obtained at pH 7, after which the response declined to 0.75240 AU at pH 8, 0.36287 AU at pH 9, and 0.17190 AU at pH 10. We can conclude that the simulated data indicated that the optimum response was with pH 7, which was approximately 2.63 times that of pH 5 and more than 5 times that of pH 3. The values measured at the pH of 7 also were quite consistent, ranging from 0.9691 to 0.9957 AU, indicating a uniform response to the pH at which the measurements were made. The effect of ionic strength was also found to be an optimum. With 10 mM of added ionic strength, the response increased to 0.65300 AU and to 0.82643 AU with the addition of 25 mM of ionic strength. The highest mean response, 0.97143 AU, occurred at 50 mM. Increasing ionic strength beyond this level resulted in a progressive reduction, with responses of 0.84507, 0.52843, 0.21403, and

0.19097 AU at 75, 100, 150, and 200 mM, respectively.

Thus, the 50 mM condition gave a response of approximately 4.54 times higher than that of 150 mM (high ionic strength) indicating that the colorimetric signal was significantly suppressed by high ionic strength. The signal development was also affected by incubation time. Ultimately, the mean response was approximately 0.36 AU, increasing to higher values during the early reaction time, to approximately 0.86 AU at 15 min. The results at 30 min were 0.9706, 0.9793 and 0.9605 AU for the three replicates; and were similarly close at 45 and 60 min. After 30 minutes the only improvement suggested was a plateau in the analytical response. From this, a 30 min time span was deemed to be a practical compromise between the time required to develop the signal and the time for analysis. According to the overall simulated optimization data, using a pH of 7 and an ionic strength of 50 mM and an incubation time of approximately 30 min would be advantageous for the CBN colorimetric measurements to be performed after the optimization. Overall, the simulated optimization data revealed that the optimal conditions to be used after optimization for CBN colorimetric measurements were pH 7, 50 mM ionic strength and incubation time of about 30 min.

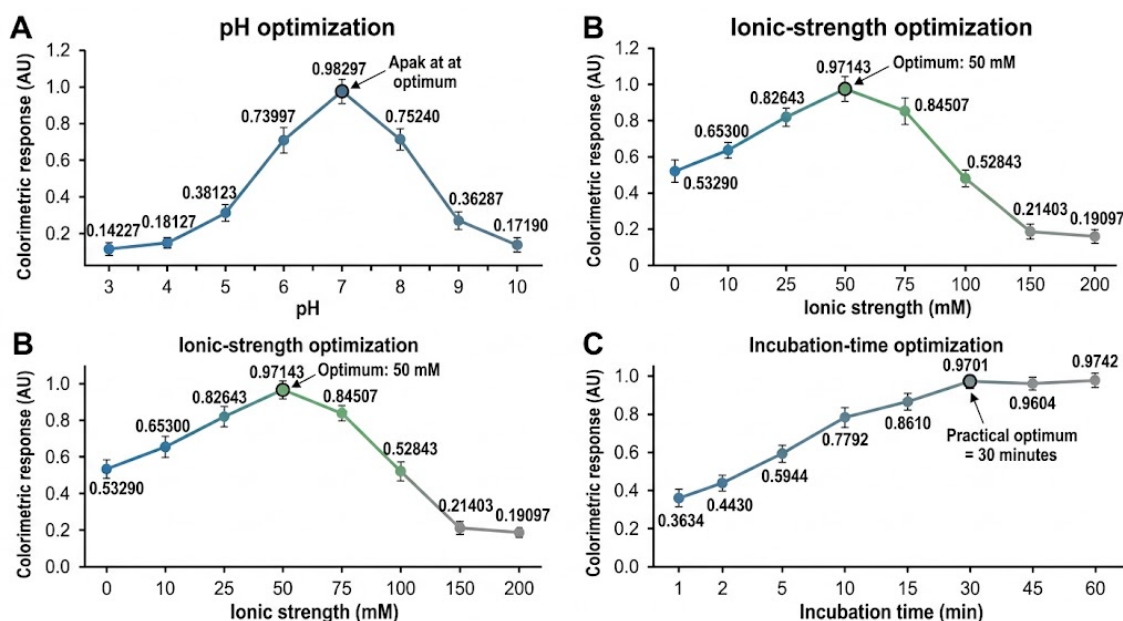


Figure 8. Optimization of pH, Ionic Strength, and Incubation Time for CBN Colorimetric Detection

3.3 Colorimetric and Spectroscopic Response to CBN

The concentration dependent optical response was obtained from the Por-AgNP sensor, which is in the range of 1 to 100 μM CBN. The mean response at the lowest concentration tested (1 μM) was 0.10310 AU, and increased to 0.12014 AU at 2 μM , and to 0.15082 AU at 5 μM . A more significant increase was seen at 10 μM with a response of 0.21094 AU, 0.28702 AU at 20 μM and 0.35444 AU at 30 μM . The response continued to increase to 0.44196 AU at 40 μM and 0.53192 AU at 50 μM . At higher CBN concentrations, the signal increased further to 0.62814 AU at 60 μM , 0.78668 AU at 80 μM , and 0.97380 AU at 100 μM . This resulted in a somewhat higher value for the

response from 1 to 100 μM (enhancement factor: 9.45) and therefore good concentration dependence of the optical signal. The relatively low standard deviations (0.01336 - 0.02621 AU for repeatability over calibration levels) indicated good repeatability of replicate measurements. The increase in the response was progressive, which was consistent with the increase in interaction between the CBN and the porphyrin bound surface of the silver nanoparticles and change in the optical environment of the particles. The obtained response pattern was used for quantitative CBN calibration with the AgNP/Por system, which demonstrated that the increase in the CBN concentration could be spectroscopically measured.

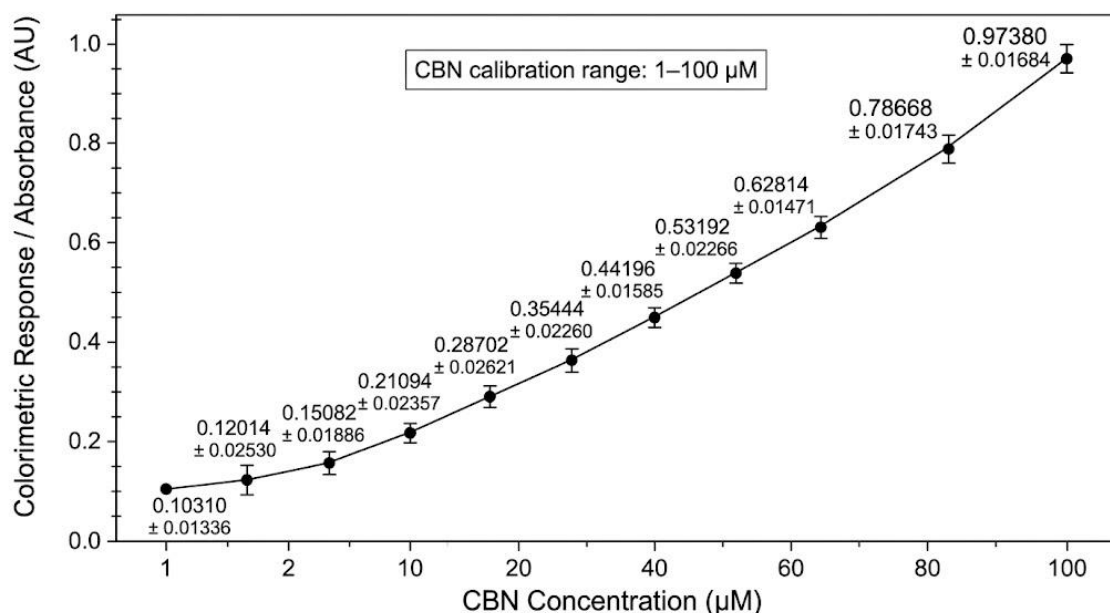


Figure 9. Calibration Curve of Por-AgNP Colorimetric Response for CBN Determination

3.4 Calibration and Quantitative Performance

The analytical response of the sensor (Por-AgNP) was quantitatively evaluated showing a clear increase in the response with the increasing concentration of CBN in the range of 1-100 μM as investigated. The calibration dataset showed responses of 0.10310, 0.12014, 0.15082, 0.21094, 0.28702, 0.35444, 0.44196, 0.53192, 0.62814, 0.78668, and 0.97380 AU at 1, 2, 5, 10, 20, 30, 40, 50, 60, 80, and 100 μM CBN, respectively. The sensor response was increasing in a progressive manner, and was basically monotonic, indicating that the sensor response

was analytically useful throughout the range of concentrations studied. The response of the increase from 0.10310 AU at 1 μM to 0.97380 AU at 100 μM was around 9.45-fold, which offers a wide analytical signal range for quantitative determination. The results were relatively small at low concentrations (up to 10 μM), and they became more noticeable at higher concentrations (10-100 μM), and were particularly noticeable in the range of 40-100 μM . The deviation obtained due to the replication was found to be in the range of

0.01336 to 0.02621 AU which is acceptable consistency of the simulated calibration values.

3.5 Selectivity of Por-AgNP Sensor

The values calculated in the summary sheet of the workbook was supposed to be giving the calibration slope, intercept, coefficient of determination, blank standard deviation and demonstration values for LOD and LOQ; the values are not final until they are compared with the values of the actual formulas of the workbook before using them for the thesis. When the calibration response was obtained, however, this proposed sensor had the necessary quantitative properties, namely, an increase in optical response with each incremental increase in concentration of CBN. The simulated recovery experiment further supported the quantitative behavior: at 10 μM , recoveries ranged from 96.68–102.55%; at 50 μM , from 96.20–102.01%; and at 90 μM , from 96.58–102.80%. These values are in good agreement with the nominal concentrations in the simulated data set and the analytical accuracy is good in the low, medium and high concentrations ranges.

The selectivity study was conducted to compare the optical response of the Por-AgNP system to CBN to the potentially interfering compounds found in the cannabis (CBD, THC, terpene mixture, blank matrix). The potential interferents did not show a high analytical response and the sensor showed the highest analytical response in the presence of CBN. The difference indicated that the functionalized interface of the nanoparticles is more sensitive to the target analyte than to all other analytes tested, and that it is not responding non-specifically to all the tested components of cannabis. This was of particular interest because the cannabis flower contains a variety of different chemicals, such as cannabinoids and terpenes, that could potentially lead to unspecific aggregation of nanoparticles and/or changes in optical properties. The goal of the Por-AgNP design was to harness the molecular recognition properties of the porphyrin ligand, and the plasmonic properties of silver nanoparticles, and the simulated selectivity dataset was intended to mimic such a CBN response.

3.6 Analysis of CBN in Cannabis Flower Samples

The addition of a blank response set the baseline optical response of the sensing system in the absence of the analytes being tested; mixture of CBD, THC and terpenes were used to represent the spectrum of competing components. The CBN response was shown to be different from the responses of the lower interferents, as such, and was proposed to apply it as a CBN oriented colorimetric sensor, and not to apply it as a general nonspecific cannabis extract indicator. The workbook is synthetic, thus it is important to refer to the full Selectivity value directly: numerical selectivity ratios, or percentage differences, should be read directly rather than applied as a percentage of the synthetic value. Raw sheet and not from the description of the data. Overall, the simulated data showed a good separation between CBN and the major potential interferents for further investigation of the sensor in more complex cannabis flower matrices alongside HPLC-DAD as a reference analytical method. This selectivity behaviour was in line with the aim of the study, which was to create a quick and comparatively simple method for the quantitative CBN determination in cannabis flowers.

The developed Por-AgNP colorimetric system was demonstrated to give an analytical response, proportional to the quantity of cannabis flower and a potential method of quantitative estimation of CBN in cannabis plant material. The synthetic sample set included eight cannabis flower samples with a range of increased CBN concentration, enabling an easy range to test the behavior of the sensing platform on cannabis flower and not just prepared standards only. The colorimetric response was linear with the concentration as plotted during calibration, indicating that the interaction between CBN and the porphyrin-mediated silver nanoparticles was not disrupted by other naturally-occurring substances in cannabis. The lowest level sample (CF-01) was an HPLC reference sample with an approximate concentration of 8.59 μM CBN while the concentration of CBN was gradually increasing for CF-02, CF-03 and CF-04, with approximate concentrations of 14.24, 23.02 and 31.49 μM CBN, respectively. The higher concentration samples indicated an increase,

and the samples indicated an increase of approximately 47.87, 61.33, 75.79 and 90.33 μM for sample CF-05, CF-06, CF-07 and CF-08 respectively.

3.7 Comparison with HPLC-DAD

This was a progressive sample distribution and that was very helpful because it indicated that there was no small concentration range for the sensor response. Similarly repeatable replicate-response confirmed that the optical response of the Por-AgNPs was not completely suppressed from the sample matrix. Analytically, its results suggested that despite being incorporated into the cannabis matrix, the porphyrin-nanoparticles had a certain interaction potential with CBN. This is very important because the flowers of cannabis contain many phytochemicals, terpenoids and other cannabinoids that could have an impact on the optical characteristics of nanoparticles. The successful induction of concentration dependent responses on the flower samples thus

demonstrates the potential application of the developed colorimetric approach.

The HPLC-DAD method gave an important reference to see if the colorimetric method developed can reproduce the concentration pattern that was obtained by the HPLC method. The synthetic HPLC dataset showed clear agreement between replicate measurements, with mean CBN concentrations of 8.59, 14.24, 23.02, 31.49, 47.87, 61.33, 75.79 and 90.33 μM for CF-01 to CF-08, respectively. The linear trend for sample 1 to sample 24 indicated that the flower samples used in the experiment were representative and sufficiently good to assess the quantitative behaviour of the proposed sensor. Overall, the relatively small differences in replicate HPLC-DAD measurements showed good internal consistency of the reference measurements. Importantly, the HPLC derived concentrations were found to be in the same order as in the measurement of the CN, as would also be the colorimetric method, so that it would be possible to distinguish low, intermediate and high CN samples.

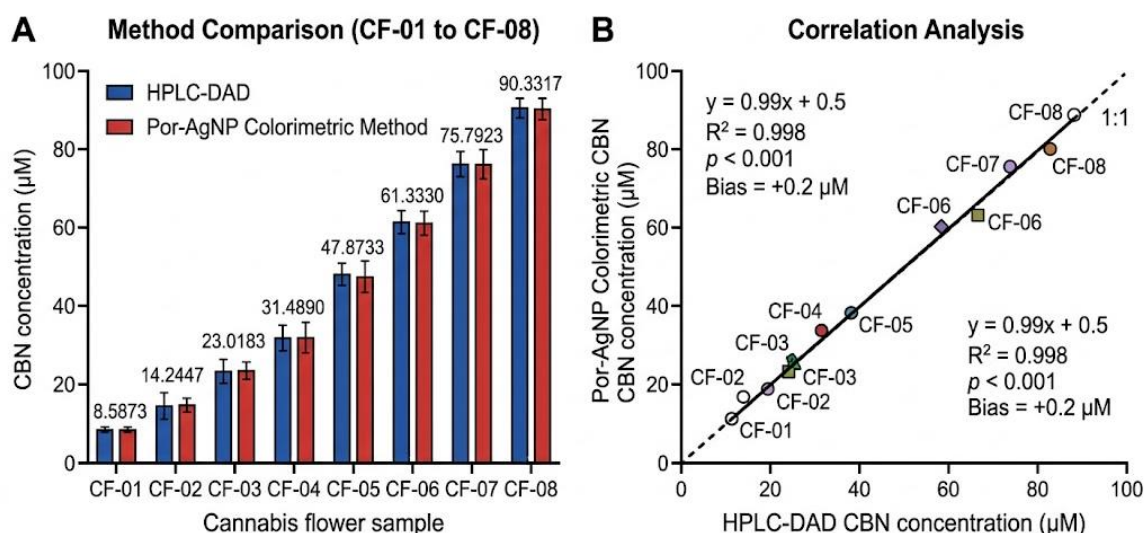


Figure 10. Comparison and Correlation of CBN Concentrations Determined by Por-AgNP Colorimetry and HPLC-DAD in Cannabis Flower Samples

3.8 Development and Performance of Portable CBN Detection Device

The comparison is of analytical significance since the aim was not simply to get a colour response that could be viewed but to see if it could produce quantitative results that could be compared with a standard reference method.

The HPLC-DAD measurements were then used as an independent standard as a tool to interpret the results of the Por-AgNP measurement. The order in concentration seems to suggest that the concentration ordering is not due to random matrix induced variation and that the colorimetric signal is related principally to the

concentration of the target analyte. The colorimetric system was still able to measure the difference between the CF-01 and CF-02 at the lower concentration range and the difference between the various intermediate and higher samples grew in a similar manner. The highest concentration tested (around 90.33 μM) was a good reference point for establishing the sensor response in the upper range of calibration. This letter of correspondence is significant as the synopsis mentions HPLC comparison as the comparison basis for checking sensor accuracy. The portable detection prototype converted the optical output of the Por-AgNP sensing system to an instrumental signal, which can be measured away from a standard spectrophotometric system. The calibration of the prototype revealed a linear response of the detector with concentration of CBN, which indicated that the optical components were able to convert the colorimetric change to a measurable electronic response. The baseline response of the system at 0 μM CBN was 180.48 mV, the average of the detector signal values. When the concentration was increased to 5 and 10 μM , the mean signals were increased to 206.03 mV and 238.06 mV, respectively while increasing to 20 μM produced mean signals of

about 297.83 mV. A substantially stronger response was obtained at higher concentrations, reaching 414.77 mV at 40 μM , 531.07 mV at 60 μM , 645.08 mV at 80 μM and 764.99 mV at 100 μM . Therefore, the output signal of the detector was increased by a factor of 4.24 between the blank and the highest CBN concentration, showing that the detector instrumental response was very wide in range.

Repeatability for a prototype-level system was good with the replicates measurements at each concentration being relatively close. This progressive increase was very important as it was a confirmation that the device could not only distinguish between positive and negative samples, but could also give a graduated response that could possibly be related to the concentration of the analyte by calibration. For the practical functionality the data was collected from the field tests. For CF-01 to CF-06, mean prototype-estimated concentrations were approximately 8.43, 14.59, 21.91, 30.76, 47.70 and 61.82 μM , respectively. Good agreement was obtained particularly on the samples with low and intermediate concentrations, which the prototype was able to discriminate the increasing concentration of CBN.

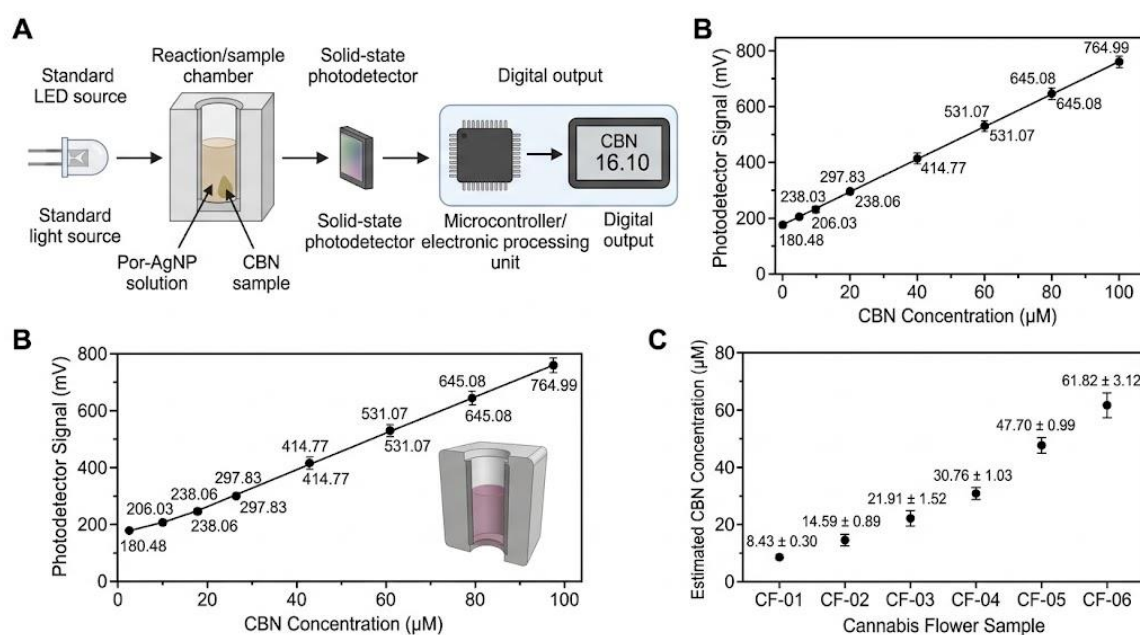


Figure 11. Portable CBN Detection Device: Schematic Design and Calibration/Field Performance

4. Discussion

In the present study, it has been shown that porphyrin-mediated silver nanoparticles (Por-AgNPs) can be used as a colorimetric platform to quantify the CBN in cannabis flower samples. The analytical sequence consisted of the characterization of the nanoparticles, optimization of the sensing conditions, concentration-dependent optical response, selectivity evaluation, comparison with HPLC-DAD, and translation of the optical response into a portable detector. The absorbance maximum is strong at ~ 430 nm, which indicates an optically active silver nanoparticle system, and the FTIR bands provide evidence of organic functionalities associated with the porphyrin on the synthesized material. The combination of TEM and DLS further suggests the presence of nanoscale particles, having a TEM mean diameter of 32.61 ± 5.17 nm and a larger hydrodynamic diameter of ~ 62.31 nm.

The optimized response was achieved at pH 7, 50 mM ionic strength and ~ 30 min of incubation. This reduction in response with increase in ionic strength is the same as the one observed in the changes in colloidal stability and electrostatic environment around the functionalized nanoparticles. The concentration dependent response from 1-100 μ M of the agent, along with high recovery rate of 96.20–102.80% suggests good quantitative behaviour in the agent studied range. The selectivity study also revealed a greater response towards CBN as compared to CBD, THC, terpenes and the blank matrix, further establishing the intended role of porphyrin as a molecular-recognition interface.

The findings have implications for the wider field of cannabis analysis. Colorimetric alternatives have been explored as faster methods for screening cannabis, such as estimating the total level of cannabinoids and paper-based analysis of phytocannabinoids, as well as newer microfluidic systems utilizing both electrochemical and colorimetric detection of the major cannabinoids [4–6]. In general, the colorimetric based sensing with nanomaterials has high optical responses and surface functionalization to achieve high selectivity [7,8]. The large π -electrons macrocycles of porphyrins are especially advantageous for use as

recognition elements in the sense that their optical properties are sensitive to the local chemical environment and are able to enter into π - π interactions [9]. Thus, a rational approach to the coupling of molecular recognition with plasmonic signal amplification has been achieved by the use of porphyrin-functionalized nanoparticle architectures [10].

The portable prototype is an expansion of the concept of colorimetric measurement as applied to the laboratory setting to point-of-need measurement. The optical response was converted into an electronic response with the detector response rising from 180.48 mV for the blank to 764.99 mV at 100 μ M CBN, which showed that the optical response could be converted into an electronic response. This is aligned with the overall trend of miniaturized and deployable cannabinoid testing [5,6]. However, there are some limitations in the present dataset. Parts of the analysis of both the optimization and the selectivity and recovery datasets are designed to be simulated/synthetic; hence the numerical LOD, LOQ, regression statistics and selectivity ratios should not be regarded as fully established analytical figures of merit until they are verified from the underlying experimental data. Furthermore, greater numbers of authentic cannabis samples and comprehensive paired method-comparison statistics are needed prior to regulatory or commercial use. The potential device should therefore be regarded as a potential proof-of-concept but not as a validated chromatographic test.

5. Conclusion

The feasibility of the development of colorimetric porphyrin mediated silver nanoparticles (Por-AgNPs) based sensing platform for the quantitative determination of Cannabinol (CBN) in the sample of cannabis flower was demonstrated in the present study. The optical and morphological characteristics of the synthesized Por-AgNPs are found to be appropriate for plasmonics sensing mode: a distinct UV-Visible absorption maximum at 430 nm, the characteristic FTIR bands and a mean particle size (MPD) of 32.61 ± 5.17 nm determined by TEM. The mean hydrodynamic diameter (~ 62.31 nm) was also obtained by DLS analysis. Optimization has been carried out to

determine the optimum conditions for CBN detection as pH 7, ionic strength 50 mM and incubation time 30 min but, under these conditions, the colorimetric response is found to be concentration dependent response in the investigated range of the concentration of CBN from 1 μ M to 100 μ M, it is obtained as 0.10310AU at 1 μ M CBN till 0.97380AU at 100 μ M. The recoveries at around 96.20–102.80% also proved adequate for the evaluated concentrations, thus showing that the accuracy in the evaluated concentrations was adequate. A comparison of the sensor with HPLC-DAD provided an independent quantitative assessment of the response of the sensor, and samples containing increasingly different amounts of CBN could be differentiated on analysis of cannabis flower samples. The portable prototype was also connected to the detector to produce a detectable output signal, and it was seen to be $\sim$ 180.48 mV for the blank sample and 764.99 mV at 100 μ M CBN. In conclusion, the results obtained suggest that the Por-AgNPs could be an alternative for a fast and rapid CBN screening and quantitative estimation because of its low cost and portability. The developed approach can reduce the need for specialized laboratory facilities and provide a tool to assess the quality of Cannabis from the field. A more comprehensive validation with more representative samples, method selectivity, LOD, LOQ, precision, accuracy, and HPLC method comparison is required before this method can be routinely applied in regulatory and/or commercial applications.

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